PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP 03/12219	International filing date *(day/month/year)* 03.11.2003	Priority date *(day/month/year)* 20.12.2002
International Patent Classification (IPC) or both national classification and IPC C12P1/02		
Applicant UNILEVER PLC et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17.06.2004	22.03.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Mueller, F Telephone No. +49 89 2399-7722

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/12219

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-34 as originally filed

Sequence listings part of the description, Pages

1 as originally filed

Claims, Numbers

1-11 as originally filed

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-11
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item I

Basis of the report

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 97/02343 A
D2: WO 99/37782 A
D3: WO 99/37673 A

D1 describes the use of the AFP-type III HPLC 12 protein as an additive in products for preventing or inhibiting ice recrystallization, see e.g. claim 1. For its recombinant expression a yeast expression system is used, see e.g. claims and p.7, l.11 ff. and p. 31 ff., p.35 l. 1 ff. The glycosylation status and the productivity is also discussed on page 28, Table 3 of present application. It is therefore considered that not all mutant strains, which are discussed in the present application, are useful for the producing of an increased amount of unglycosylated AFP. D1 furthermore refers on page 7, l.11-21 to an expression method for AFP III, HPCL 12, which allows its expression at large scale and at low cost.

D2, a document also in the field of AFPs, describes AFPs of the Gramineae family which are expressed recombinantly (E. coli) and which have significant ice-recrystallisation inhibition properties, see e.g. p.9, l.8 ff. Furthermore D2 describes that the recombinant expressed (in E. coli) non-glycosylated antifreeze protein from Lolium perenne retains its recrystallization inhibition activity, see p. 35, l.5 ff. and that glycosylation of the AFP is not required for its activity, see p.33, l.1 ff. and claim 6.

D3, a document also in the filed of AFP, AFPs from Lichen, postulates that modifications e.g. of the glycosylation status do not influence the ice recrystallisation inhibition properties, see p. 5, l.6 ff. and claim 6.

The disclosure of D1, see e.g. claim 1 and p.6, l.27 -34, is considered to provide evidence that AFP-III HPLC 12 have an influence in the ice recrystallization process and therewith is contradictory to the RI inhibition results provided in Table 2 of the present application.

The problem to be solved of the present application with respect to the cited prior art (D1-D3) is considered to provide AFPs fractions with improved ice recrystallization inhibition properties. However AFPs fractions with and without modified glycosylation pattern are already provided in the prior art and are used for influencing the ice recrystallization and therefore it is considered that the posed problem is already convincingly solved by the prior art (see D2,D3 and D1). The requirements for an inventive step for claims 1-11 are thus not fulfilled (Article 33(3) PCT).

Re Item VII
Certain defects in the international application

The application should be self-contained. Therefore the term "incorporated by reference" found on page 33, l.2 in the description does not fulfil the requirements of the PCT-Guidelines c-II, 4.17.

Re Item VIII
Certain observations on the international application

The subject-matter of claim 1 is not clear. No reference in the wording of the claims is made to the antifreeze protein sequence (structural feature, origin) which is to be produced and no structural features are given of the fungal expression system for achieving a less glycosylated protein fractions, see also Table 3 of present application. Therefore claim 1 does not meet the requirements of Article 6 PCT, PCT-Guidelines C-III, 4.7.

The subject-matter of claim 10 is not clear (Article 6 PCT). The claimed recombinant type III antifreeze protein AFP is not defined by structural features.